REMARKS

Entry of the foregoing, further and favorable reconsideration of the subject application are respectfully requested, in light of the following remarks pursuant to and consistent with 37 C.F.R. §1.116.

By the foregoing amendment, a new Abstract of the Disclosure, which can be found at the end of this Amendment on a separate sheet of paper, has been added as requested by the Examiner. Claims 27, 36-39, 42-44 and 53 have been amended to further clarify Applicants' invention. No new matter has been added.

I. Objections to the Specification

The Examiner has objected to the specification for lacking an Abstract . Applicants submit herewith an Abstract of the Disclosure on a separate sheet of paper.

II. Claim Objections

The Examiner has objected to claim 36 as being in improper dependent form. To expedited prosecution in the subject application, Applicants have rewritten claim 36 in independent form. Accordingly, this objection is rendered moot.

III. Rejections Under 35 U.S.C. § 112

Claims 27-29, 32-35 and 51-53 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point our and distinctly claim the subject matter which Applicants regard as the invention. This rejection is respectfully traversed.

The Examiner has stated that "[i]t is unclear where the metes and bounds of ergosterol biosynthesis lie. Biosythesis and metabolism in organisms are complex, intertwined processes" and that one could argue that glucose was an intermediate product since it is involved in most biosynthetic processes. Applicants respectfully disagree.

Applicants submit that the phrase "intermediate products" is well known and understood in the art to mean a molecular species that is formed from reactants and which further reacts to form the products of the reaction. The American Heritage College dictionary defines an intermediate as "a substance formed as a necessary stage in the manufacture of a desired end product." The Merriam-

Webster Collegiate Dictionary defines an intermediate as a species "formed in a reaction as an intermediate step between the starting materials and the final product."

Therefore, Applicants respectfully request withdrawal of the rejection of claims 27-29, 32-35 and 51-53 under 35 U.S.C. § 112, second paragraph.

Claims 27-53 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point our and distinctly claim the subject matter which Applicants regard as the invention. This rejection is respectfully traversed.

The tHMG gene described in the specification is an altered form of Basson et al.'s HMG1 gene. Page 7, lines 13-20, and page 9, lines 3-6, of the specification clearly describe that tHMG is an altered form of the HMG1 gene of Basson et al. as well as the specific alteration in the gene. As for the SAT1 and ARE1 genes, Applicants submit that ARE1 in Yang et al. (1996) corresponds to SAT1 in Yu et al. (1996). This is so stated in Yu et al. (*J. Biol. Chem.*, 271(39):24157-63 (1996)) (enclosed herewith). See the Noted Added in Proof on page 24163 above the list of references (second column). Therefore, there is no confusion with regard to the terms "t-HMG" and "SAT1."

The remaining rejections under 35 U.S.C. § 112, second paragraph, have been rendered moot in light of the amendments to the claims.

Accordingly, Applicants respectfully request withdrawal of the rejection of claims 27-53 under 35 U.S.C. § 112, second paragraph.

Claims 27-31, 33-36 and 43-53 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed had possession of the claimed invention. This rejection is respectfully traversed.

It is well settled that to comply with the description requirement, "it is not necessary that the application describe the claimed invention in ipsis verbis; all that is required is that it reasonably convey to persons skilled in the art that, as of the filing date thereof, the inventor had possession of the subject matter later claimed by him." *In re Edwards*, 196 U.S.P.Q. 465 (C.C.P.A. 1978). Further, determining whether the written description requirement is satisfied requires reading the specification in light of the knowledge possessed by the skilled artisan. Such knowledge can be established by, *inter alia*, reference to patents and publications available to the public prior to the

filing date of the application. *See In re Alton*, 76 F.3d 1168 (Fed. Cir. 1996); *In re Lange*, 644 F.2d 856 (C.C.P.A. 1981).

Applicants submit that it is not necessary to disclose the sequences of the genes of the invention because the sequences are publicly known. Pages two and three of the specification disclose citations for each of the genes. Further, the sequences for the genes are readily available at the National Center for Biotechnology Information (GenBank) (ERG1-assession No. M64994; SAT1- assession No.U55383; HGM1-assession No. M22002; ERG9- assession No.X59959). Therefore, the subject specification provides the skilled artisan with sufficient disclosure.

Accordingly, Applicants respectfully request withdrawal of the rejection of claims 27-31, 33-36 and 43-53 under 35 U.S.C. § 112, first paragraph.

Claims 32 and 37-42 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. This rejection is respectfully traversed.

A deposit of the plasmids is unnecessary, because one of skill in the art can readily reproduce them without undue experimentation, using methods taught by the specification. See the specification, for example at pages 20-21, for specific details as to how to produce these plasmids. See also MPEP 2404.02: "Applicant may show that a deposit is not necessary even though specific biological materials are required to practice the invention if those biological materials can be made or isolated without undue experimentation."

The Examiner has stated that the starting materials must be publicly available in order that the plasmids of the invention need not be deposited.

To expedite prosecution, Applicants submit that the yeast strain AH22 is widely used in the art and is publicly available (ATCC No. 38626; strain S288C is publicly available also (ATCC No. 26108)). Further, starting materials pUC19 (ATCC No. 37254) and Yep13 (ATCC No. 37115) are publicly available. Vector YdpU is a pUC9 (ATCC No. 37252) based vector that is described in Berben et al. (1991).

Accordingly, Applicants respectfully request withdrawal of the rejection of claims 32 and 37-42 under 35 U.S.C. § 112, first paragraph.

IV. Rejections Under 35 U.S.C. § 102

Claim 53 has been rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Sanders et al. (EP 0 486 290 A2). This rejection is rendered moot in light of the amendment to claim 53. Sanders et al. does not teach each and every element of the claimed invention.

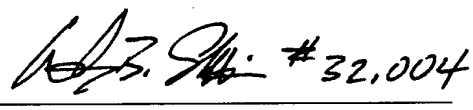
Accordingly, Applicants respectfully request withdrawal of the rejection of claim 53 under 35 U.S.C. § 102(b).

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

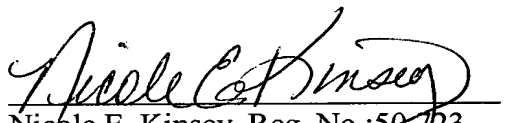
In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that prosecution of this application may be expedited.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,



Anthony J. Zelano, Reg. No. 27,969
Attorney for Applicant(s)



Nicole E. Kinsey, Reg. No.: 50,723
Agent for Applicant(s)

MILLEN, WHITE, ZELANO
& BRANIGAN, P.C.
Arlington Courthouse Plaza 1, Suite 1400
2200 Clarendon Boulevard
Arlington, Virginia 22201
Telephone: (703) 243-6333
Facsimile: (703) 243-6410

Date: November 4, 2002

VERSION WITH MARKINGS TO SHOW CHANGES MADE

27. (Amended) A method of producing ergosterol or one or more intermediate products of its biosynthesis, comprising,

a) designing a plasmid, into which the following genes are inserted:

i) t-**HMG**, an HMG-Co-A-reductase gene,
ERG9, a squalene synthetase gene,
SAT1, an Acyl-CoA: sterol-acyl transferase gene, and
ERG1, a squalene epoxidase gene,

or

ii) t-**HMG**, an HMG-Co-A-reductase gene, and
ERG9, a squalene synthetase gene,

or

iii) t-**HMG**, an HMG-Co-A-reductase gene, and
SAT1, an acyl-CoA: sterol-acyl transferase gene,

or

iv) t-**HMG**, an HMG-Co-A-reductase gene, and
ERG1, a squalene epoxidase gene,

or

v) **ERG9**, a squalene synthetase gene, and
SAT1, an acyl-CoA: sterol-acyl transferase gene,

or

vi) **ERG9**, a squalene synthetase gene, and
ERG1, a squalene epoxidase gene,

or

vii) **SAT1**, an acyl-CoA: sterol-acyl transferase gene, and
ERG1, a squalene epoxidase gene,

or

viii) one of the genes [that is mentioned in i)] <u>selected from the group consisting of ERG9, SAT1 and ERG1</u>,

b) transforming a microorganism with a plasmid mentioned in i) to vii), or, simultaneously

or in succession, with two or more of the plasmids mentioned in viii), and

c) culturing the transformed microorganism under conditions in which it produces ergosterol and an intermediate product of ergosterol biosythesis.

36. (Amended) A yeast strain **S. cerevisiae** AH22 [that contains one or more of the genes that are mentioned in i) of claim 27] comprising at least one gene selected from the group consisting of t-**HMG**, an HMG-Co-A-reductase gene, **ERG9**, a squalene synthetase gene; **SAT1**, an Acyl-CoA sterol-acyl transferase gene; and **ERG1**, a squalene epoxidase gene.

37. (Amended) The plasmid YEpH2, which comprises [an] the **ADH**-promoter, [a] the **t-HMG** gene, and [a] the **TRP**-terminator, as shown in Fig. 1.

38. (Amended) The plasmid YDpUHK3, which comprises [an] the **ADH**-promoter, [a] the **t-HMG** gene, [a] the **TRP**-terminator, [a] the gene for kanamycin resistance and [a] the **ura3** gene, as shown in Fig. 2.

39. (Amended) The plasmid pADL-SAT1, which comprises [a] the **SAT1** gene and the **LEU2** gene of YEp13, as shown in Fig 3.

42. (Amended) [The method according to claim 41, wherein the intermediate product is a sterol with a 5,7-diene structure] A method for producing an intermediate sterol product with a 5,7-diene structure in the biosynthesis of ergosterol, comprising transforming a microorganism with a plasmid according to claim 37, and culturing the transformed microorganism under conditions in which it produces said intermediate sterol product.

43. (Amended) An expression cassette that comprises a **t-HMG** gene [flanked by] operatively linked to an **ADH**-promoter and a **TRP**-terminator, and an **SAT1** gene [flanked by] operatively linked to an **ADH**-promoter and a **TRP**-terminator.

44. (Amended) An expression cassette that comprises a **t-HMG** gene [flanked by] operatively linked to an **ADH**-promoter and a **TRP**-terminator, and an **SAT1** gene [flanked by]

operatively linked to an **ADH**-promoter and a **TRP**-terminator, and an **ERG9**-gene [flanked by] operatively linked to an **ADH**-promoter and a **TRP**-terminator.

53. (Amended) A method for producing ergosterol or one or more intermediate products of its biosynthesis, comprising expressing in a microorganism a plasmid which comprises the following genes:

i) t-**HMG**, an HMG-Co-A-reductase gene,
ERG9, a squalene synthetase gene,
SAT1, an Acyl-CoA: sterol-acyl transferase gene, and
ERG1, a squalene epoxidase gene,

or

ii) t-**HMG**, an HMG-Co-A-reductase gene, and
ERG9, a squalene synthetase gene,

or

iii) t-**HMG**, an HMG-Co-A-reductase gene, and
SAT1, an acyl-CoA: sterol-acyl transferase gene,

or

iv) t-**HMG**, an HMG-Co-A-reductase gene, and
ERG1, a squalene epoxidase gene,

or

v) **ERG9**, a squalene synthetase gene, and
SAT1, an acyl-CoA: sterol-acyl transferase gene,

or

vi) **ERG9**, a squalene synthetase gene, and
ERG1, a squalene epoxidase gene,

or

vii) **SAT1**, an acyl-CoA: sterol-acyl transferase gene, and
ERG1, a squalene epoxidase gene,

or

viii) one of the genes [that is mentioned in i)] selected from the group consisting of ERG9, SAT1 and ERG1,

and isolating the expressed ergosterol or intermediate products of its biosynthesis.

ABSTRACT

A process is described for the production of ergosterol and its intermediate products, using recombinant yeast and plasmids for transformation of yeast.